# New dendritic Gd-based contrast agents with PEG cores for tumor imaging

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## Introduction

Intravascular Gd-based contrast media have prolonged angiographic effect and unique potential in MRI to quantitate microvascular characteristics which has been proven by many pre-clinical studies with a variety of macromolecular formulations.[1,2] To overcome existing drawbacks associated with different designs (e.g. size polydispersity, incomplete elimination, potential immunogenicity), an array of new dendritic contrast agents was synthesized (Fig. 1) by introduction of two polylysine dendrons to both ends of a polyethyleneglycol (PEG) via divergent synthesis, followed by surface lysine decoration with Gd chelates. Their relaxivity and blood pharmacokinetics were studied. Two selected compounds were tested for tumor characterization in an experimental human breast cancer model implanted in athymic rats.

## Materials and Methods

Relaxivity measurements were conducted at 0.25 T PRAXIS II relaxometer and a 2 T Omega CSI-II MRI scanner (Bruker). Blood pharmacokinetic parameters were measured in normal female Sprague-Dawley rats using a two-compartment distribution model. Athymic rats (4-5 weeks, female) implanted with MDA-MB-435 cells ($4x10^6$) underwent dynamic contrast-enhanced MRI (3D-SPGR) with chosen contrast macromolecules. Tumor microvascular permeability ($K^{ps}$) values were calculated using a uni-directional two-compartmental tissue model and SAAM II software.[3]

## Results and Discussion

Synthesis: PEG-core dendritic polylysines with different generations (32 and 64 amino groups available for Gen4 and Gen5 respectively) were synthesized including PEG3400-Gen4, PEG3400-Gen5, PEG6000-Gen4 and PEG12000-Gen4. The dendrimer synthesis was conducted by a stepwise divergent approach using a PEG bisamine (MW 3400 or 6000 or 12000) as a macro core and di-t-Boc-L-lysine as the monomer. Tri-t-butyl-DO3A-MetGly (Schering AG) or DTPA was converted to its N-hydroxysuccinimide ester respectively, subsequently reacted in excess with surface primary amino groups of the PEG-core dendrimers, then underwent t-butyl deprotection in trifluoroacetic acid, finally chelated with Gd(III) in aqueous solution. A series of dendrimeric conjugates thus obtained were purified chromatographically and characterized by size-exclusion HPLC, ICP-AES, elemental analysis (and NMR spectra prior to Gd chelation). High degree of molecular weight monodispersity (Mw/Mn = 1.07 or less) was obtained for these compounds. Further efforts are ongoing to achieve improved efficiency in the dendrimer decoration step.

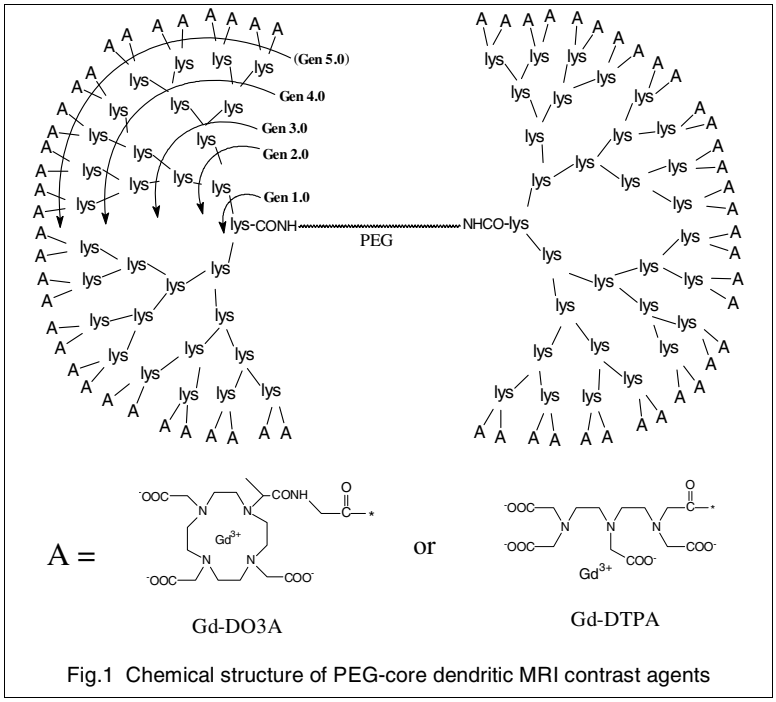


Fig.1 Chemical structure of PEG-core dendritic MRI contrast agents

*In vitro* relaxivity and blood pharmacokinetics. At 37 °C, these new contrast agents exhibited the T1 relaxivity approximately 2-fold (0.25 T) or 3-fold (2 T) as much as the clinically used small molecular contrast agents, *e.g. Magnevist* or *Dotarem*. No difference was observed for the T1 relaxivity in pure water and plasma, indicating the absence of plasma protein binding for this class of contrast agents. Blood curves ($\Delta R1$ vs. time) of these compounds in normal rats (n = 3 for each) were obtained by dynamic MRI (dose 0.04 mmol Gd/Kg). Calculated pharmacokinetic parameters include blood half-life ($t_{1/2}\alpha$, distribution phase; $t_{1/2}\beta$, elimination phase) and volume of distribution ($V_d$). PEG3400-Gen5-(Gd-DTPA), PEG12000-Gen4-(Gd-DTPA) and PEG12000-Gen4-(Gd-DO3A) all showed intravascular characteristics with mono-exponential kinetics in blood (Table 1).

Tumor imaging. PEG3400-Gen5-(Gd-DTPA) and PEG12000-Gen4-(Gd-DTPA) were applied for tumor characterization in MDA-MB-435 tumor-bearing nude rats (n = 3 for each) using dynamic MR imaging at a dose of 0.04 mmol Gd/Kg. Significant difference (by unpaird, two-tailed t-tests) was found in the $K^{ps}$ values (ml $min^{-1}$ $100cc^{-1}$, mean±SD) between tumor (0.018 ± 0.004) and muscle (0.004 ± 0.007) ($p< 0.05$) for PEG3400-Gen5-(Gd-DTPA), and also between tumor (0.081± 0.047) and muscle (0.0 ± 0.0) ($p< 0.05$) for PEG12000-Gen4-(Gd-DTPA).

***Table 1 Properties of PEG-core dendritic MRI contrast agents***

| Contrast agent | MW (kDa) | Appr. MW * (kDa) | No. of Gd | T1 relaxivity ($mM^{-1}$ $s^{-1}$)** 0.25T | T1 relaxivity ($mM^{-1}$ $s^{-1}$)** 2 T | Blood $t_{1/2}$ (min) | $V_d$ (ml/Kg) |
|---|---|---|---|---|---|---|---|
| **PEG12000-Gen4-(Gd-DTPA)** | **20.1** | **132** | **8.0** | **8.6** | **9.1** | **40.0** | **135** |
| **PEG6000-Gen4-(Gd-DTPA)** | **14.2** | **82** | **7.5** | **8.7** | **10.0** | **5.9 (α) 43.2(β)** | **211** |
| **PEG3400-Gen5-(Gd-DTPA)** | **18.4** | **106** | **12.9** | **10.7** | **9.7** | **89.1** | **89** |
| **PEG12000-Gen4-(Gd-DO3A)** | **24.3** | **133** | **13.4** | **9.7** | **nd** | **33.2** | **148** |
| **PEG6000-Gen4-(Gd-DO3A)** | **19.9** | **78** | **15.2** | **9.9** | **nd** | **nd** | **nd** |
| **Gd-DTPA (*Magnevist*)** | **0.55** | **na** | **1** | **5.1** | **2.9** | **13.1** | **242** |

*Apparent molecular weight compared to protein standards. ** At 37 °C

## Conclusion

This new class of PEG-core dendritic MRI enhancers showed great potential as blood pool agents with molecular weights even less than 25 kDa, also showed 3-fold relaxivity as much as *Magnevist*, and were able to differentiate xenograft human breast cancer and normal musculature in athymic rats by DCE-MRI technique, represented by a Gd-based PEG12000-Gen4 construct. Further, their blood pharmacokinetics can be rationally tuned by adjusting PEG-core size, generation number of the dendritic polylysine, number and electric charge of the covalently-attached Gd chelate.

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